Attenuation of renal fibrosis after unilateral ischemia reperfusion may require a multi-target approach

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Background and Aim
Incomplete recovery from severe AKI is an important pathway to persistent and progressive CKD and recent studies have suggested that even complete functional recovery from AKI is associated with an increased risk for CKD development [1]. Understanding the mechanisms underlying the progression from acute to chronic renal injury is the focus of recent research in the field [2]. We previously optimized a mouse model of AKI to CKD by unilateral ischemia-reperfusion (UIRI) without contralateral nephrectomy, with development of moderate renal fibrosis and significant long-term inflammation. To validate this model for use in therapeutic intervention studies, 3 experimental treatments were tested: administration of 1) recombinant human CCN3 (rhCCN3; CCN2/CTGF antagonist), 2) TGFβ1 neutralizing antibody (2011) and 3) dexamethasone (corticosteroid).

Materials and Methods
- Male C57Bl/6 mice underwent 21 min of UIRI at 36°C body temperature, the contralateral kidney was left undisturbed. A heated surgical pad was used to maintain body temperature during ischemia, continuously monitored with a rectal feedback thermometer.
- 9 treatment groups (n=4/group, q) were included:
  1. a sham group
  2. an untreated UIRI group
  3. rhCCN3 (5 µg/kg, daily)
  4. vehicle (PBS, daily)
  5. antibody to TGFβ1 (0.5 mg/kg, every other day starting d0)
  6. antibody to TGFβ1 (0.5 mg/kg, every other day starting d8)
  7. vehicle (PBS, every other day)
  8. dexamethasone (10 mg/kg, 3 consecutive days, then every other day)
  9. vehicle (PBS, 3 consecutive days, then every other day).

Results
Fibrosis-related parameters
UIRI without treatment induced a significant reduction of renal mass in
- a sham group
- an untreated UIRI group
- rhCCN3 (5 µg/kg, daily)
- vehicle (PBS, daily)
- antibody to TGFβ1 (0.5 mg/kg, every other day starting d0)
- antibody to TGFβ1 (0.5 mg/kg, every other day starting d8)
- vehicle (PBS, every other day)
- dexamethasone (10 mg/kg, 3 consecutive days, then every other day)
- vehicle (PBS, 3 consecutive days, then every other day).

- Three weeks after UIRI renal fibrotic outcome was determined by gene expression analysis (µPCR) of collagen I, TGFβ, CCN2 (CTGF), CCN3, α-SMA and TNFα.

- Renal morphology and fibrosis were evaluated on ischemic kidney tissue stained with Masson’s trichrome stain and collagen I immunostaining.

- Additional immunostainings on ischemic kidney tissue were performed to determine the proliferative response (Ki67), inflammation (F4/80 macrophage/monocyte) and the amount of fibroblasts in the ischemic kidney and quantified using the AstraView image analysis software.

Conclusions
Despite the earlier proven benefits of TGFβ antagonism and CCN3 treatment on the development of fibrosis in other animal models, neither treatment (at doses demonstrated to be effective in more mild injury models) showed effect in the URI model. Anti-inflammatory suppression by dexamethasone attenuated fibrotic gene expression in the ischemic kidney. We speculate that the natural course of renal demise after URI is very robust and is highly likely to require a multi-target therapeutic approach.

References
3. Three weeks after UIRI significant upregulation of expression of fibrosis-related genes Col (17 fold), TGFβ (13 fold), CCN2 (4 fold), CCN3 (10 fold), α-SMA (20 fold) and TNFα (11 fold) was observed in untreated animals (black bars) as compared to sham (white bars). Neither treatment with rhCCN3 or anti-TGFβ had an effect on the expression of the fibrosis-related genes. Dexamethasone treatment, however, did induce a significant lower expression of Col I and CCN2, as compared to both untreated (black bars) and vehicle-treated animals (red stripped bars), indicating a diminished pro-fibrotic response upon dexamethasone treatment.
4. A significantly increased amount of macrophage staining was present in the untreated group as compared to sham (white circles). Neither treatment with rhCCN3, anti-TGFβ or dexamethasone had an effect on the amount of macrophage staining in the ischemic kidney.

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Poster Design: Dirk De Weerdt
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